

Synthesis, characterization and antimicrobial activity of norfloxacin based acetohydrazides

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Abstract: The drug resistance phenomenon in microbes is resulting in the ineffectiveness of available drugs to treat the infections. Thus, there is a continued need to discover new molecules to combat the drug resistance phenomenon. Norfloxacin is a fluoroquinolone antibiotic that is used for the treatment of urinary tract infections. In this research work, norfloxacin is structurally modified by hybridizing with a range of substituted acetohydrazidic moieties through a multistep reaction. The first step involves the coupling of norfloxacin 1 with methyl chloroacetate followed by the treatment with hydrazine hydrate to result in corresponding acetohydrazide 3. A range of substituted benzaldehydes were reacted with the acetohydrazide to form the targeted series of norfloxacin derivatives 4a-i. The final compounds were screened for antimicrobial activity. Among the tested compounds, 4c, 4d, 4e and 4f displayed better antifungal activity against *F.avenaceum*, while compound 4c and 4e were active against *F. bubigeum*.

Keywords: Norfloxacin, acetohydrazides, antimicrobial agents, Schiff bases.

INTRODUCTION

Infectious diseases are becoming uncontrolled due to the development of drug resistance in bacteria resulting in the ineffectiveness of antibiotics (Levin-Reisman *et al.*, 2017). Fluoroquinolone family of drugs has been widely used for more than 50 years for the treatment of bacterial infections (Tulkens *et al.*, 2019). Norfloxacin (a fluoroquinolone antibiotic) is effectively used to treat bacterial infections caused by Gram-positive and Gram-negative bacteria (Chierentin and Salgado 2016). The antibacterial activity of fluoroquinolone derivatives is due to the inhibition of supercoiling of bacterial DNA-gyrase which stop the bacterial DNA synthesis resulting in bacterial cell death (Towle *et al.*, 2018). The side-effects attributed to norfloxacin are gastroenteritis, cartilage damage and vomiting. These side effects are serious concern to human beings all over the world and require alternate antibacterial strategies (Hu *et al.*, 2017; Zhang *et al.*, 2018). Unfortunately, most of the first-generation drugs have become ineffective due to the development of resistance in microbes for antibiotics (Baym *et al.*, 2016).

On the other hand, schiff bases are well known for their versatile biological activities, such as, antioxidants (Al Zoubi *et al.*, 2016), antifungal (Wei *et al.*, 2019), antibacterial (Mondal *et al.*, 2015), antidiabetic (Okoli

and Modise 2018), antimalarial (Aggarwal *et al.*, 2018), antiproliferative (Parlak *et al.*, 2019), anti-inflammatory (Murtaza *et al.*, 2017), antipyretic (Gangrade and Karande 2018) and antiviral (Wang *et al.*, 2018) activities.

Keeping in view the biological significance of norfloxacin and schiff bases, the titled norfloxacin based schiff bases 4a-i were prepared and screened for antimicrobial activity.

MATERIALS AND METHODS

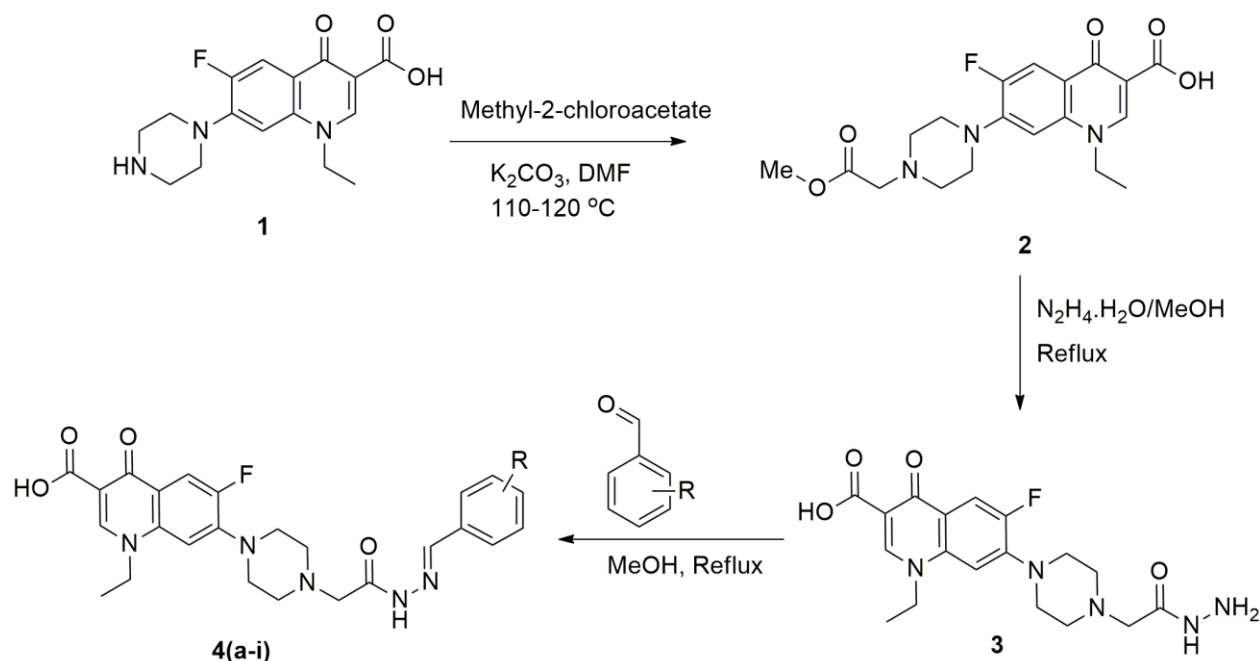
General experimental part

All the chemicals were purchased from Alfa Aesar. ¹H NMR spectra's of the synthesized compounds were recorded at Bruker instrument at 600 MHz. Chemical shifts are reported in ppm.

General procedure for the synthesis of norfloxacin derivatives 4a-i

A mixture of norfloxacin 1 (1.57 mmol), methyl-2-chloroacetate (2.04 mmol) and DMF (20 ml) was stirred at 110-120°C for 4 hrs. Thin layer chromatography (TLC) was used to monitor the progress of chemical reaction. On completion of the reaction, the precipitates of the product were obtained by adding excess cold water. The crude product 2 was purified by column chromatography to get 45% yield. The product 2 (1.0g) was dissolved in MeOH and refluxed with equimolar hydrazine hydrate for 3.5

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Scheme 1: Synthetic lay-out for titled compounds 4a-i.

hours. The ppts of compound 3 were obtained by adding 10% dil. HCl. Finally, a mixture of compound 3 (1.28 mmol), equimolar quantities of substituted benzaldehydes and 4-5 drops of glacial acetic acid were refluxed in methanol to get ppts of 4a-i. Column chromatography was performed for the purification of all compounds (Scheme 1) (table 1).

Biological activity

Antimicrobial activity by well diffusion method

Antimicrobial activity of norfloxacin derivatives is checked through well diffusion method against six bacterial strains in which two were Gram positive (*Bacillus cereus* & *Staphylococcus aureus*) and four were Gram negative (*Salmonella enterica*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Aeromonas hydrophila*). The antimicrobial activities against two fungal strains (*Fusarium avenaceum* and *Fusarium bubigeum*) were also checked. The stock solution of each compound (10 mg/ml) was prepared in DMSO (Dimethyl Sulfoxide) as solvent. Then 0.5 McFarland Standard (2 x 10⁸ colony forming unite CFU/ml) Inoculum of Bacterial and Fungal strain was uniformly spread on ager surface in Patri plate with sterile cotton swab. Then wells were made into this ager with cork borer of (8mm) for delivering drugs. About 50µl of each compound (10mg/ml stock solution) was added in relevant well and 50µl DMSO as vehicle was added into one well as a negative control. The 25µg/ml cefixime was added in a well as positive control to confirm antimicrobial activity (Dadpe *et al.*, 2018). After drug delivery plates were sealed with parafilm and placed there for 2 hours for proper drug diffusion. These plates were incubated for 24 hours at 37°C temperature and

normal pressure of 1atm. After 24 hours, incubation plates were examined and inhibition zone were seen and diameter of these inhibition zones were measured with antibiotic zone measuring scale.

RESULTS

Chemistry

The characterization of final compounds has been carried out with the help of NMR and mass spectrometry. The results are described in table 2.

Biological activity

The results of antimicrobial evaluation are described in table 3.

DISCUSSION

Chemistry

The competent synthetic methodology for norfloxacin based acetohydrazides involves the coupling with methyl chloroacetate in dimethylformamide as solvent. It was noted that moisture free conditions are suitable for the *N*-alkylation step. The ester moiety (compound 2) was reacted with hydrazine hydrate to form corresponding hydrazide. The workup of this reaction is crucial for further processing of the scheme of work. The mixture was precipitated in dil. HCl which enabled the removal of unreacted hydrazine, traces of which could lead in formation of impurities in next step. Finally, compound 3 was reacted with substituted benzaldehydes to get final series of acetohydrazides. The synthesis was confirmed with NMR and LCMS.

Table 1: Structural parameters and reaction time for the synthesized norfloxacin derivatives 4(a-i).

| S. No. | Compound | R | Reaction Time (h) | S. No. | Compound | R | Reaction Time (h) |
|--------|----------|-------------------------|-------------------|--------|----------|------------------------------------|-------------------|
| 1. | 4a | 2-OH | 12.0 | 6. | 4f | 4-N(CH ₃) ₂ | 20.0 |
| 2. | 4b | H | 12.0 | 7. | 4g | 4-OH | 14.0 |
| 3. | 4c | 4-OH-3-OCH ₃ | 16.0 | 8. | 4h | 3-OCH ₃ | 16.0 |
| 4. | 4d | 4-Cl | 14.0 | 9. | 4i | 3-OH | 16.0 |
| 5. | 4e | 2-Cl | 12.0 | | | | |

Table 2: Spectral data of compounds 4a-i

| Compound | M.P. (°C) | Yield (%) | Characterization Data |
|----------|-----------|-----------|--|
| 4a | 225 | 77 | ¹ H NMR (DMSO- <i>d</i> ₆ , 600 MHz) δ: 1.41 (s, 3H, CH ₃), 2.72 (s, 2H, NCH ₂ CO), 2.90 (br-s, 1H, OH), 3.25 (s, 4H, Pip), 3.37 (s, 4H, Pip), 4.51 (s, 2H, NCH ₂ CH ₃), 6.87-6.92 (m, 2H, Ar-H), 7.13-7.29 (m, 2H, Ar-H), 7.48 (d, <i>J</i> = 7.32 Hz, 1H, Ar-H), 7.87 (d, <i>J</i> = 13.14 Hz, 2H, Ar-H), 8.45 (s, 1H, CH=N), 8.83 (s, 1H, CONH), 11.16 (s, 1H, COOH); ¹³ C NMR (DMSO- <i>d</i> ₆ , 150 MHz) δ: 14.9, 49.8, 49.9, 50.2, 53.0 (2C), 60.4, 107.6(2C), 117.1, 117.2, 119.1, 120.4, 130.4, 132.5, 138.0, 146.4, 149.0, 149.2, 152.9, 157.9 (2C), 166.8, 167.6, 176.9. LC-MS (m/z): calcd: 494 [M-H] ⁻ , 496 [M+H] ⁺ , 518 [M+Na] ⁺ , 534 [M+K] ⁺ ; found: 494 [M-H] ⁻ , 496 [M+H] ⁺ , 518 [M+Na] ⁺ , 534 [M+K] ⁺ . |
| 4b | 276 | 71 | ¹ H NMR (DMSO- <i>d</i> ₆ , 600 MHz) δ: 1.41 (t, <i>J</i> = 6.26 Hz, 3H, CH ₃), 2.70 (s, 2H, NCH ₂ CO), 3.32-3.34 (m, 4H, Pip), 3.37 (m, 4H, Pip), 4.51 (d, <i>J</i> = 7.08 Hz, 2H, NCH ₂ CH ₃), 7.11-7.14 (m, 1H, Ar-H), 7.42-7.44 (m, 2H, Ar-H), 7.65 (d, <i>J</i> = 6.49 Hz, 1H, Ar-H), 7.70-7.71 (m, 1H, Ar-H), 7.86 (d, <i>J</i> = 6.92 Hz, 1H, Ar-H), 7.90 (dd, <i>J</i> = 13.33, 5.77 Hz, 1H, Ar-H), 8.24 (s, 1H, Ar-H), 8.84 (d, <i>J</i> = 1.94 Hz, 1H, CH=N), 11.22 (s, 1H, CONH), 11.35 (s, 1H, COOH); LC-MS (m/z): calcd: 478 [M-H] ⁻ , 480 [M+H] ⁺ , 502 [M+Na] ⁺ , 518 [M+K] ⁺ ; found: 478 [M-H] ⁻ , 480 [M+H] ⁺ , 502 [M+Na] ⁺ , 518 [M+K] ⁺ . |
| 4c | 285 | 65 | ¹ H NMR (DMSO- <i>d</i> ₆ , 600 MHz) δ: 1.40 (t, <i>J</i> = 7.10 Hz, 3H, CH ₃), 2.69 (s, 2H, NCH ₂ CO), 2.78 (br-s, 1H, OH), 3.35 (s, 4H, Pip), 3.63 (s, 3H, OCH ₃), 3.78 (s, 4H, Pip), 4.48 (d, <i>J</i> = 6.87 Hz, 2H, NCH ₂ CH ₃), 6.81 (d, <i>J</i> = 8.09 Hz, 1H, Ar-H), 7.06 (d, <i>J</i> = 6.82 Hz, 1H, Ar-H), 7.11 (d, <i>J</i> = 6.88 Hz, 1H, Ar-H), 7.39 (s, 1H, Ar-H), 7.87 (d, <i>J</i> = 13.28 Hz, 1H, Ar-H), 8.12 (s, 1H, Ar-H), 8.79 (s, 1H, CH=N), 9.63 (s, 1H, CONH), 11.21 (s, 1H, COOH); LC-MS (m/z): calcd: 524 [M-H] ⁻ , 526 [M+H] ⁺ , 548 [M+Na] ⁺ , 564 [M+K] ⁺ ; found: 524 [M-H] ⁻ , 526 [M+H] ⁺ , 548 [M+Na] ⁺ , 564 [M+K] ⁺ . |
| 4d | 255 | 81 | ¹ H NMR (DMSO- <i>d</i> ₆ , 600 MHz) δ: 1.41 (t, <i>J</i> = 6.98 Hz, 3H, CH ₃), 2.75 (s, 2H, NCH ₂ CO), 3.25 (s, 4H, Pip), 3.38 (s, 4H, Pip), 4.51 (t, <i>J</i> = 6.57 Hz, 2H, NCH ₂ CH ₃), 7.16 (dd, <i>J</i> = 17.70, 8.3 Hz, 1H, Ar-H), 7.48 (d, <i>J</i> = 8.29 Hz, 2H, Ar-H), 7.70 (d, <i>J</i> = 8.33 Hz, 1H, Ar-H), 7.72 (d, <i>J</i> = 8.44 Hz, 1H, Ar-H), 7.93 (s, 1H, Ar-H), 8.22 (s, 1H, Ar-H), 8.85 (s, 1H, CH=N), 8.86 (s, 1H, CONH), 9.94 (s, 1H, COOH); LC-MS (m/z): calcd: 512 [M-H] ⁻ , 514 [M+H] ⁺ , 536 [M+Na] ⁺ , 552 [M+K] ⁺ ; found: 512 [M-H] ⁻ , 514 [M+H] ⁺ , 536 [M+Na] ⁺ , 552 [M+K] ⁺ . |
| 4e | 261 | 82 | ¹ H NMR (DMSO- <i>d</i> ₆ , 600 MHz) δ: 1.42 (t, <i>J</i> = 6.09 Hz, 3H, CH ₃), 2.63 (s, 2H, NCH ₂ CO), 2.88-2.89 (m, 4H, Pip), 3.43 (s, 4H, Pip), 4.53 (t, <i>J</i> = 7.65 Hz, 2H, NCH ₂ CH ₃), 7.16 (s, 1H, Ar-H), 7.21 (d, <i>J</i> = 7.36 Hz, 1H, Ar-H), 7.39-7.42 (m, 2H, Ar-H), 7.43-7.46 (m, 1H, Ar-H), 7.50-7.51 (m, 1H, Ar-H), 7.93-7.96 (m, 1H, Ar-H), 8.88 (s, 1H, CH=N), 10.31 (s, 1H, CONH), 11.98 (s, 1H, COOH); LC-MS (m/z): calcd: 512 [M-H] ⁻ , 514 [M+H] ⁺ , 552 [M+K] ⁺ ; found: 512 [M-H] ⁻ , 514 [M+H] ⁺ , 552 [M+K] ⁺ . |
| 4f | 272 | 57 | ¹ H NMR (DMSO- <i>d</i> ₆ , 600 MHz) δ: 1.41 (t, <i>J</i> = 6.23 Hz, 3H, CH ₃), 2.40 (s, 2H, NCH ₂ CO), 2.76 (s, 6H, N(CH ₃) ₂), 2.84 (s, 4H, Pip), 3.01 (s, 4H, Pip), 4.51-4.52 (m, 2H, NCH ₂ CH ₃), 6.71-6.73 (m, 3H, Ar-H), 6.77 (d, <i>J</i> = 8.45 Hz, 1H, Ar-H), 7.16 (d, <i>J</i> = 7.34 Hz, 1H, Ar-H), 7.21 (d, <i>J</i> = 7.17 Hz, 1H, Ar-H), 7.68 (d, <i>J</i> = 8.44 Hz, 1H, Ar-H), 8.88 (s, 1H, CH=N), 9.59 (s, 1H, CONH), 11.19 (s, 1H, COOH); LC-MS (m/z): calcd: 521 [M-H] ⁻ , 523 [M+H] ⁺ , 545 [M+Na] ⁺ , 561 [M+K] ⁺ ; found: 521 [M-H] ⁻ , 523 [M+H] ⁺ , 545 [M+Na] ⁺ , 561 [M+K] ⁺ . |

| Compound | M.P. (°C) | Yield (%) | Characterization Data |
|----------|-----------|-----------|--|
| 4g | 269 | 63 | ¹ H NMR (DMSO- <i>d</i> ₆ , 600 MHz) δ: 1.40 (t, <i>J</i> = 6.96 Hz, 3H, CH ₃), 2.69 (s, 2H, NCH ₂ CO), 2.79 (br-s, 1H, OH), 3.16 (s, 4H, Pip), 3.34 (s, 4H, Pip), 4.48 (s, 2H, NCH ₂ CH ₃), 6.80 (q, <i>J</i> = 26.55, 4.22 Hz, 2H, Ar- <i>H</i>), 7.11 (d, <i>J</i> = 6.06 Hz, 1H, Ar- <i>H</i>), 7.49 (d, <i>J</i> = 8.52 Hz, 1H, Ar- <i>H</i>), 7.55 (d, <i>J</i> = 8.22 Hz, 1H, Ar- <i>H</i>), 7.85-7.87 (m, 1H, Ar- <i>H</i>), 8.13 (s, 1H, Ar- <i>H</i>), 8.82 (s, 1H, CH=N), 9.73 (s, 1H, CONH), 11.18 (s, 1H, COOH); ¹³ C NMR (DMSO- <i>d</i> ₆ , 150 MHz) δ: 14.6, 49.6 (2C), 49.9, 52.6, 52.7, 60.2, 106.0, 107.2, 111.5, 116.2(2C), 119.4, 125.6, 129.6 (2C), 137.7, 146.0, 148.6, 152.5, 159.4, 166.4, 167.2, 176.5. LC-MS (m/z): calcd: 494 [M-H] ⁻ , 496 [M+H] ⁺ , 518 [M+Na] ⁺ , 534 [M+K] ⁺ ; found: 494 [M-H] ⁻ , 496 [M+H] ⁺ , 518 [M+Na] ⁺ , 534 [M+K] ⁺ . |
| 4h | 213 | 61 | ¹ H NMR (DMSO- <i>d</i> ₆ , 600 MHz) δ: 1.41 (t, <i>J</i> = 7.13 Hz, 3H, CH ₃), 2.70 (s, 2H, NCH ₂ CO), 3.20 (s, 4H, Pip), 3.37 (s, 3H, OCH ₃), 3.65 (s, 4H, Pip), 4.51 (d, <i>J</i> = 7.05 Hz, 2H, NCH ₂ CH ₃), 6.98 (td, <i>J</i> = 7.57, 2.53 Hz, 1H, Ar- <i>H</i>), 7.14 (d, <i>J</i> = 7.16 Hz, 1H, Ar- <i>H</i>), 7.24-7.26 (m, 1H, Ar- <i>H</i>), 7.31 (s, 1H, Ar- <i>H</i>), 7.34-7.37 (m, 1H, Ar- <i>H</i>), 7.89-7.93 (m, 2H, Ar- <i>H</i>), 8.21 (s, 1H, CH=N), 8.85 (s, 1H, CONH), 11.23 (s, 1H, COOH); LC-MS (m/z): calcd: 508 [M-H] ⁻ , 510 [M+H] ⁺ , 532 [M+Na] ⁺ , 548 [M+K] ⁺ ; found: 508 [M-H] ⁻ , 510 [M+H] ⁺ , 532 [M+Na] ⁺ , 548 [M+K] ⁺ . |
| 4i | 288 | 75 | ¹ H NMR (DMSO- <i>d</i> ₆ , 600 MHz) δ: 1.41 (t, <i>J</i> = 6.90 Hz, 3H, CH ₃), 2.70 (s, 2H, NCH ₂ CO), 2.78 (br-s, 1H, OH), 3.19 (s, 4H, Pip), 3.36 (s, 4H, Pip), 4.50 (d, <i>J</i> = 6.08 Hz, 2H, NCH ₂ CH ₃), 6.80-6.83 (m, 2H, Ar- <i>H</i>), 7.07 (d, <i>J</i> = 7.14 Hz, 1H, Ar- <i>H</i>), 7.11 (d, <i>J</i> = 7.37 Hz, 2H, Ar- <i>H</i>), 7.14 (s, 1H, Ar- <i>H</i>), 7.22-7.26 (m, 1H, Ar- <i>H</i>), 8.84 (s, 1H, CH=N), 9.92 (s, 1H, CONH), 11.31 (s, 1H, COOH); LC-MS (m/z): calcd: 496 [M+H] ⁺ , 518 [M+Na] ⁺ , 534 [M+K] ⁺ ; found: 496 [M+H] ⁺ , 518 [M+Na] ⁺ , 534 [M+K] ⁺ . |

Table 3: Antimicrobial activity of norfloxacin derivatives 4a-i, Inhibition zone were mm at 0.5mg/ml drug concentration in 8mm well size

| Microbial Strains | Inhibition Zone (mm) | | | | | | | | | | | |
|----------------------|----------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----------------------------|
| | 1 | 2 | 4a | 4b | 4c | 4d | 4e | 4f | 4g | 4h | 4i | Positive Control (Cefixime) |
| Bacterial strains | 18 | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | 12 |
| <i>B. cereus</i> | 18 | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | 12 |
| <i>S. aureus</i> | 36 | -ve | -ve | -ve | -ve | -ve | 20 | 23 | 20 | 25 | 21 | 18 |
| <i>S. enterica</i> | 31 | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | 15 |
| <i>E. coli</i> | 35 | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | 10 |
| <i>P. aeruginosa</i> | 23 | 27 | 23 | 26 | -ve | -ve | -ve | -ve | -ve | -ve | -ve | 20 |
| <i>A. hydrophila</i> | 29 | 28 | 22 | 24 | 30 | -ve | -ve | -ve | -ve | -ve | -ve | 15 |
| Fungal Strains | 1 | 2 | 4a | 4b | 4c | 4d | 4e | 4f | 4g | 4h | 4i | Positive Control |
| <i>F. avenaceum</i> | 30 | -ve | -ve | -ve | 25 | 16 | 22 | 23 | -ve | -ve | -ve | 30 |
| <i>F. bubigeum</i> | 13 | -ve | -ve | -ve | 12 | -ve | 13 | -ve | -ve | -ve | -ve | 12 |

In ¹H NMR, piperazine-protons appeared in the form of two groups *i.e.*, 2.84-3.35 and 3.01-3.78 shift value ranges. Similarly Gan *et al.* observed the piperazine protons in the form of two groups having chemical shift values in the range of 2.39-2.43 ppm and 3.53-3.63 ppm (Gan *et al.*, 2018). Carboxylic acid functionality appeared at 9.94-11.98 ppm. In addition, CH=N group appeared at 8.21-8.88 shift value. Salve *et al.* also reported that CH=N group appeared in the range of 7.82-8.34 ppm (Salve *et al.*, 2017). Moreover, methoxy substitutions in compounds 4c and 4h were observed at 3.63 and 3.37 ppm respectively. Methoxy functionality was observed by Abbasi *et al.* in the range of 3.35-3.78 ppm (Abbasi *et al.*, 2017). Moreover, the proposed structures were confirmed by appearance of M⁺ peaks in LC-MS (table 2).

Biological Activity

Biological activity of norfloxacin derivatives is checked by well diffusion method and results are summarized in table 3. Norfloxacin derivatives are reported in literature as antimicrobial agents (Wang *et al.*, 2019). In order to overcome the drug resistance, it is very common to modify the chemical structures of previously in-use drugs. Marin *et al.* modified norfloxacin and the derivatives exhibited significant antimicrobial potential (Marin *et al.*, 2018). In another study, 7 or 3-substituted norfloxacin derivatives appeared as potent bioactive agents (Oniga *et al.* 2018). Guo *et al.* identified norfloxacin derivatives as potentially active against *S. aureus* (Guo *et al.* 2019) Our study indicated that some of these derivatives have antimicrobial potential against selective bacterial as well

as fungal strains., its inhibition zone against *Bacillus cereus* is 18mm, against *Salmonella enterica* is 31mm, against *Escherichia coli* is 35mm and against *Pseudomonas aeruginosa* is 23mm, against *Aeromonas hydrophila* is 29mm. It is also effective against both fungal strains, *Fusarium avenaceum* with inhibition zone of 30mm and *Fusarium bubigeum* with inhibition zone of 13mm. Compound 2, 4a and 4b were found to be potent against only two bacterial strains *P. aeruginosa* with inhibition zone of 27mm, 23mm, 26mm and *A. hydrophilia* with inhibition zone of 28mm, 22mm, 24mm respectively. They were also inactive against both fungal strains (*F. avenaceum* & *F. bubigeum*). Compound 4c was active against one bacterial strain *Aeromonas hydrophilia* with zone of inhibition of 30mm and also active against *F. avenaceum* 25mm and *F. bubigeum* 12mm. Compound 4e, 4f, 4g, 4h and 4i all were found potent against *Staphylococcus aureus*. Their zone of inhibition was 20mm, 23mm, 20mm, 25mm and 21mm respectively. From these five compounds, 4e and 4f were also potent against fungi. 4e was active against both fungi *F. avenaceum* & *F. bubigeum* with 22mm and 13mm inhibition zone respectively while 4f was potent against only *F. avenaceum* strain with 23mm inhibition zone (table 3).

CONCLUSION

In this research work, we have reported a new series of norfloxacin derivatives having acetohydrazide substitution at piperazinyl ring. The antimicrobial results have indicated that piperazine ring plays a crucial role in the biological activity. Compound 2 and 4b demonstrated better activity than norfloxacin against *P. aeruginosa*, while compounds 4e, 4f, 4g, 4h and 4i were more active than cefixime against *S. aureus*. The results would be useful for devising more effective antimicrobial agents.

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